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QUALITY COMPOSITION AND BIOLOGICAL SIGNIFICANCE OF THE BANGLADESHI AND CHINA GINGER (*ZINGIBER OFFICINALE* ROSC.)

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ABSTRACT

The essential oil of *Zingiber officinale* Rosc. was extracted from China and Bangladeshi varieties and yielded 0.21% and 0.23% by hydro-distillation method on fresh weight basis respectively. Fifteen compounds were identified and quantified by GC-MS. The major constituents of China and Bangladeshi ginger essential oils were zingiberene 38.10% and 41.49%, β -phellandrene 12.0% and 9.92%, α -citral 11.48% and 9.76%, α -curcumene 9.22% and 11.58%, camphene 5.94% and 4.60%, β -bisabolene 4.39% and 5.0% respectively. The IC₅₀ (DPPH method) values were found 61.18 μ g/mL and 56.71 μ g/mL with the highest inhibition of 78.49% and 80.77% and the LC₅₀ values in the brine shrimp lethality cytotoxicity bioassay were found 0.4842 μ g/mL and 0.7151 μ g/mL in China and Bangladeshi ginger essential oil respectively. Both the essential oils showed significant activities against some gram positive, gram negative bacteria and fungi. The proximate composition of the China and Bangladeshi variety showed the ash (7.12 \pm 0.151, 8.15 \pm 0.18%), protein (5.47 \pm 0.19, 6.60 \pm 0.16%), crude fibre (4.32 \pm 0.10, 4.61 \pm 0.12%), carbohydrate (16.06 \pm 0.35, 18.38 \pm 0.41) and food energy (70.50 \pm 0.89, 81.74 \pm 1.01 kcal/100g.) respectively. The elemental compositions of the both varieties were found rich in Ca, Mg, Fe, Al, Se, Na and K. These results indicate the quality composition of the two varieties may find interest in spice and culinary industries as well as in medicinal preparation.

Keywords: *Zingiber officinale*, essential oil, limonene, GC/MS, mineral element, antimicrobial, antioxidant, cytotoxicity

INTRODUCTION

Spice and medicinal plants gained a more important role in agronomy production, pharmacy and exportation because of their increased use as a raw material for the pharmaceutical industry and in the everyday life (Abou et al., 2000). In recent years, research and cultivation of spice plants have been achieved with increasing interest in our country because of the production of good quality spice and also the possibility of exportation.

Zingiber officinale Rosc. commonly known as ginger (Bengali name Ada), belonging to the family Zingiberaceae of the genus *Zingiber* is comprised of some forty genera and hundreds of species. It is an important culinary aromatic spice for its long standing utility as a flavoring agent. The plant is indigenous to warm tropical climates, particularly South-Eastern Asia. It is also extensively cultivated in India, China, Japan, Australia, Africa, Mexico, Hawaii and Jamaica (Evans 1989). The plant is an erect perennial, growing from 1-3 ft. in height. The rhizomes are aromatic thick lobed branched and scaly structures with a spicy lemon-like scent. The essential oil and oleoresins extracted from ginger rhizomes are responsible for the characteristic ginger flavor and pungency. Both oil and oleoresins are used in many food items, soft drinks, beverages, pickles, and many types of medicinal preparations (Gurdip et al., 2008). The steam-volatile oil content of dried gingers of commerce ranging from about 1 to 2.5% (Anonymous 2008). The essential oil, which is mixture of terpenoid compounds is responsible for the characteristic odorants components. The most predominant important reported components are α -zingiberene, geraniol, β -bisabolene, nerol, 1,8-cineol, α -terpineol, borneol, p-cineole, α -terpineol β -phellandrene, α -curcumene, α -farnesene, β -sesquiphellandrene, camphene, neral, geranyl acetate, α -terpinene; cadina- 1,4-diene, 6-gingerol and 6-shogaol (Ghorab et al., 2010; El-Baroty et al., 2010; Moshafi et al., 2009; Zancan et al., 2002; Erler et al., 1988; Ekundayo et al., 1988; Miyazawa et al., 1988; Malek et al., 2005; Sultan et al., 2005; Pino et al., 2004; Kochhar et al., 2003). Ginger is one of the widely used spices having a long history in traditional oriental medicines as antimicrobial and antioxidant (Habsah et al., 2000), anti-inflammatory (Tjendraputra et al., 2001), anti-fungal (Ficker et al., 2003),

inhibitor of nitric oxide synthesis (Ippoushi et al., 2003), antiarrhoeal activity (Poonam et al., 2010), anticonvulsant activity (Sayyah et al., 2005), treatment of epilepsy (Kermani et al., 1988) and an agent for protecting neuronal cells from amyloid insult (Kim and Kim, 2004). The anticancer properties are reported the presence of pungent vullinoids e.g. 6-gingerol and 6-paradol, shogaols Zingerone, etc. (Shukla et al., 2006).

The chemistry of ginger has the subject of sporadic study since the early 19th century. In recent years, the considerable advances on clear understanding of the chemical composition to its quality properties have emerged. There are numerous studies on the composition and activities of ginger essential oil but the comparative study of different growing varieties and quality composition parameters are still lacking. The present paper deals with the chemistry, comparative study of antioxidant, antimicrobial properties and toxic label of ginger essential oil as well as elemental composition of the two varieties.

MATERIAL AND METHODS

Plant material

Two varieties of ginger rhizomes were collected from local supplier. One was imported from China and another one was from Bangladeshi cultivated ginger. The samples were cleaned, freed from dirt and others specimen, air-dried in room temperature, cut into small pieces and stored in airtight high-density double lined polyethylene bag for future research work.

Extraction of essential oils

Ginger was subjected to hydro-distillation using Clevenger's apparatus (Clevenger 1928) for 4 h and then dried over anhydrous sodium sulfate and stored at -20°C prior to analysis.

Physico-chemical and proximate studies

The physico-chemical and proximate studies of essential oils and Ginger powder of the two varieties were carried out with three replications by the standard methods (AOAC 2005; BP 2004; Guenther 1953).

GC-MS analysis

The essential oils of *Zingiber officinale* Rosc. rhizomes were analyzed by Electron Impact Ionization (EI) method on GC-2010 Shimadzu Gas Chromatograph, coupled to a GC-MS QP 2010 plus Shimadzu Mass Spectrometer; RTX-5 MS fused silica capillary column (Supelco Inc.) (30m x 2.5mm; 0.25 µm film thickness). Column temperature was 40°C (hold 2 min) to 220°C (hold 5 min) at the rate of 10°C/min, maintained with carrier gas helium at a constant pressure of 90 kPa (Acquisition parameters full scan; scan range 40-550 amu). Samples were injected by splitting with the split ratio 10. Mass spectra were taken at 70eV.

Identification of the compounds

The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions based on co-injection of homologous n-alkanes (C₆-C₂₄) on RTX-5 MS capillary column. Compounds were identified by comparison of their mass spectra with those of the internal reference mass spectral NIST-107 library.

Antioxidant Activity

The antioxidant activity of the essential oil on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the Brand-Williams method (Brand-Williams 1995) with some modifications. In the experiment, 2 mg of each oil was dissolved in methanol and applied by serial dilution technique which concentrations range from 1000 to 1.9531 µg/mL. Two milliliters of a methanol solution of the oil of each concentration was mixed with 3 mL DPPH-methanol solution (40 mg/L) and allowed to stand for 30 min. Then the absorbance was measured at 517 nm using an Analytic Jene Spekel 1300 UV spectrophotometer and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [1 - (\text{ABS}_{\text{sample}} / \text{ABS}_{\text{control}})] \times 100$$

Then per cent inhibitions were plotted against respective concentrations. The IC₅₀ values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph (linear regression curve). Ascorbic acid and BHT (tert-butyl-1-hydroxytoluene) a potential antioxidant were used as positive control at the concentration of 500 µg/mL-1.953 µg/mL. The experiment was performed triplicate and the results were expressed as mean ±SD with 95% confidence interval in every case.

Antimicrobial Screening

The disc diffusion method (Bauer 1966; Radovanović 2009). was used to test antimicrobial and antifungal strains against five gram positive, eight gram negative organisms and three fungi. The bacterial and fungal strains used for the experiment were collected as pure culture from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The test samples (8 mg) were made by dissolving in calculated volumes of solvents separately and applied to sterile discs (6 mm diameter) at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvents. Discs containing the test material, were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic ciprofloxacin (5 µg/disc) discs and blank discs (impregnated with solvents) were used as positive and negative control respectively. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Cytotoxicity Activity of Brine Shrimp Lethality Bioassay

In vitro lethality bioassay of the essential oils of ginger rhizome was exploited to detect cytotoxicity by the Mayer's method (Meyer et al., 1982). The eggs of Brine Shrimp was collected from local pet shops and hatched in a tank (3.8% w/v sea salt in distilled water) at 30°C in front of a lamp. Eggs were hatched within 48 hours providing large number of larvae (nauplii). The test samples (essential oil) were prepared by dissolving in DMSO (not more than 50 µL in 5 ml solution) and it was applied in 5 mL brine solution (3.8% NaCl in water) to attain concentrations of 0.078 to 40.0 µg/mL. A vial containing 50µL DMSO diluted to 5mL of brine solution was used as a control. Standard vincristine sulfate (VcS) was used as positive control at the concentration of 0.078 µg/mL to 10.0 µg/mL. Then matured shrimps (10 of each vial) were applied to all experimental and control vials. After 24 hours, the vials were inspected using a magnifying glass in front of lamp and the number of surviving nauplii in each vial were counted. The lethal concentrations of essential oil

resulting in 50% mortality of the brine shrimp (LC₅₀) from the 24 h counts and the dose-response data were calculated.

Elemental Analyses

Reagents

All reagents used were of analytical-reagent grade. Nitric acid (HNO₃) and perchloric acid (HClO₄) were used of trace metal analysis grade (E-Merck Germany). A clean laboratory and laminar-flow hood capable of producing class 100 were used for preparing solution. High purity de-ionized water having a resistivity of 17.5–18.0 MΩ/cm obtained using a Milli-Q water purification system (Millipore, Bedford, MA USA) was throughout. All solutions were stored in high-density polyethylene bottles. Plastic materials were cleaned by soaking in 10% (v/v) HNO₃ for 24h, rinsing three times with de-ionized water and dried in a class 100 laminar flow hood before use. Multi-element stock solutions containing 10 mg/L of each element were obtained from USA (Accu Trace™ Reference Standard, 125 market Street New Haven, CT06513, USA). For the determination of dissolved elements, the samples were filter through 0.45 micron filter paper, then preserved with 1.0% concentrated HNO₃ by volume (0.5 % concentrated HCl is only added for Ag). For the determination of total recoverable elements in unfiltered samples it was acidified with HNO₃ as described above. In all cases, the pH of the samples maintained and verified to be less than 2.0 prior to analysis.

Ashing procedure and Sample preparation for ICP-MS: A certain amount of moisture less sample powder was taken. Ashing procedure and subsequent samples preparation were performed as per AOAC method (AOAC 2005).

ICP-MS tuning solution: Contains 10 ppb Ba, Be, Ce, Co, In, Pb, Mg, Tl and Th for instrument tuning and verification of performance.

Metals stock standard: 10 mg/L (Reference / Traceable) of metals Al, As, Ba, Be, Bi, Cd, Ca, Cs, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Ag, Na, Sr, Tl, U, V, Zn.

Preparation of intermediate (100 µg/L) standard: Take 1.0 mL metal stock standard in a 100 mL volumetric flask and dilute up to the mark with 1% HNO₃ diluent.

Preparation of working standard for calibration: Prepare 1, 5, 10, 20 and 50 µg/L working standard from 100 µg/L intermediate standard for carrying out analysis. Take 0.5 mL for 1, 2.5 mL for 5, 5 mL for 10, 10 mL for 20 and 25 mL for 50 µg/L standard solutions from 100 µg/L intermediate standard solution in separate 50 mL volumetric flasks, dilute up to the mark with 1% HNO₃ diluent.

Instrumentation: The ICP-MS measurements were done using a Varian UltraMass™ ICP-MS system (Varian Optical Spectroscopy Instruments, Melbourne Australia). The plasma source was 99.998% argon (Carbagas 3097, Liebefeld, Bern, Switzerland). All operating parameters were under computer control, which allowed simple and fast optimization routines for different matrices. The instrument operating conditions were depicted in Table -1.

Table 1 Operating conditions of Varian ICP-MS

Parameters	Settings
<i>Plasma conditions</i>	
RF power	1.40 kW
Plasma Ar flow rate.	18.0 L min ⁻¹
Auxiliary Ar flow rate	2.25 L min ⁻¹
Sheath Gas Flow	0.20 L min ⁻¹
Nebulizer gas flow	1.0 L min ⁻¹
Sampling depth	6.50 mm
Pump Rate	5 rpm
<i>Instrument</i>	
Sampler cone: Nikel	1.0 mm orifice diameter
Skimmer cone: Nikel	0.5 mm orifice diameter

Atomic Absorption Spectrometry Method

The elements Mg, Hg, Fe, Ca, Al were analyzed by Varian Spectra AA 240 FS (Varian Inc. USA) Atomic Absorption Spectrometry. The total mercury in the powder samples (About 2.50 g) were analyzed by a Cold Vapor process (CV-AAS) at a wavelength of 254 nm. coupled with a Varian Vapor Generation (VGA) following the Varian Operation Manual. The quantification limit for the elements were 0.01 µg/kg(ppb). Powder samples were preparation as per AOAC method (AOAC 2005).

Flame Photometric Analyses

The concentration of sodium (Na⁺) and potassium (K⁺) were analyzed by Flame photometer (Jenway PFP-7, England, UK), A graded series of Standard (

ranging from 1-5 ppm) of Na⁺ and K⁺ solution were used in the serial dilution method for standard curve within linear calibration range and total quantities in samples were calculated.

Data Analysis

The results of antioxidant activity data were transformed into a straight line by means of a trend line fit linear regression analysis by MS Excel version 7 software and cytotoxicity by Biostat 2007 data analysis software. The statistical significants were calculated as p value by SPSS for windows version 13.0 software.

RESULTS AND DISCUSSION

Proximate composition

The proximate compositions of China ginger and Bangladeshi ginger are shown in **Table-2**. China ginger showed higher moisture content (80.42±0.44%) than Bangladeshi ginger (77.30±0.25%). Dry and organic matter contents of both the ginger varieties were calculated on the basis of moisture and ash values respectively. Moisture contents of both the varieties are almost similar to those already reported data (Peter et al., 1999; Govindrarajan 1982). Bangladeshi ginger had higher total ash, water soluble ash, acid insoluble ash, nitrogen, protein, crude fibre, fatty acid, Essential oil, carbohydrate and food energy than the China ginger variety. Protein, crude fibre and carbohydrate contents are the important from the nutritional point of view. As per literature, carbohydrate content reported on fresh weight basis was 7.6±0.679% (EL-Ghorab et al., 2010) which might be up to 12.30% depending on varieties (Anonymous 2008), crude fibre content of the fresh ginger reported 8.20±0.36% (EL-Ghorab et al., 2010) whereas commercial ginger reported 1.5-6% (Anonymous 2008). On the other hand protein contents were reported 7.2±0.09 and 12.40% on fresh weight basis (EL-Ghorab et al., 2010; Panwar et al., 2005). The results of protein, crude fibre and carbohydrate contents are significantly higher than the reported values. Because of high moisture contents in our samples, which affect significantly on the constituents. Fatty acid contents were found significantly higher than the earlier reported (EL-Ghorab et al., 2010; Anonymous 2008) values (1.4±0.049%, and 0.9%). Essential oil contents of Bangladeshi (0.23%) and China (0.21%) ginger variety determined on fresh weight basis. The results are between the earlier reported data which are 0.31±0.08% and 0.17 % on fresh basis (EL-Ghorab 2010; Famurewa 2011). This variation may depend upon the variety, climatic variation and geographical difference. In the case of ash content, dry ginger has been reported 6.64% and 6.1±0.05% (Peter et al., 1999; EL-Ghorab et al., 2010) which are comparatively lower values than the current experiment. High amount of water soluble ash contents indicate the presence of highly soluble minerals contents and acid insoluble ash value indicates high digestibility of Bangladeshi ginger variety. The food energy value is emphasizing a little bit high (81.74±1.01) in Bangladeshi variety comparing with the international specification (80.0 kcal) (USDA 2011).

Table-2 Proximate composition of Ginger varieties (g/100g)

Parameters	China Ginger	Bangladeshi Ginger
Moisture	80.42±0.44 ^b	77.30±0.25 ^b
Dry mater	18.10±0.90	22.69±0.25
Organic Matter	92.87±0.156	91.83±0.20
Total Ash	7.12±0.151 ^a	8.15±0.18 ^a
	1.39±0.09 ^b	1.85±0.07 ^b
Water soluble Ash	3.74±0.11 ^a	4.54±0.17 ^a
Acid in soluble Ash	1.24±0.29 ^a	1.66±0.19 ^a
Nitrogen (N ₂)	0.876±0.01 ^a	0.97±0.01 ^a
Protein	5.47±0.19 ^a	6.06±0.16 ^a
	1.07±0.01 ^b	1.37±0.01 ^b
Crude Fiber	4.32±0.10 ^a	4.61±0.12 ^a
	0.845±0.01 ^b	1.04±0.02 ^b
Fatty acid	1.13±0.04 ^a	2.24±0.05 ^a
	0.22±0.01 ^b	0.50±0.01 ^b
Essential oil	0.21±0.00 ^b	0.23±0.005 ^b
Carbohydrate	1.52±0.35 ^a	1.62±0.23 ^a
	16.06±0.35 ^b	18.38±0.41 ^b
Food Energy (kcal/100g)	37.96±1.29 ^a	50.93±0.95 ^a
	70.50±0.89 ^b	81.74±1.01 ^b

Each value represents the average value from three experiments of Mean ±SD, P<0.05 of independent sample T-test. ^a On dry weight basis, ^b on the fresh weight basis.

Physico-chemical properties of the essential oil

The physico-chemical properties of the two varieties essential oils are shown in **Table-3**. Both oils are transparent pale yellow in color with the characteristics odor, bitter taste, freely miscible in some organic solvents and also in 80% ethanol. The essential oil of ginger had specific gravities 0.9033 ± 0.002 and 0.9176 ± 0.002, refractive indexes [η]^{30°C} 1.487 ± 0.00 and 1.4877 ± 0.00, optical rotations [α]_D^{26°C} -39.41° ± 0.01 and -38.24° ± 0.04, acid value (mg KOH/g) 7.71±0.31 and 8.53±0.14, ester values (%) 34.37±0.19 and 37.26±0.81 for China and Bangladeshi ginger varieties respectively. The current experimental data are in good agreement with Govindranath (1982).

Table 3 Physico-chemical properties of the essential oil of Ginger variety

Characteristics	China Ginger	Bangladeshi Ginger
Appearance (30°C)	A homogeneous, transparent, pale yellow liquid and lighter than water	
Odor and taste	A spicy odor with bitter taste	
Miscibility and solubility	Insoluble in water but freely miscible in chloroform, carbon tetrachloride, diethyl ether, n-hexane, 100% alcohol, 5.0 volumes in 80% alcohol and 0.1 volumes in 90% alcohol.	
Specific gravity at 30°C	0.9033 ± 0.002	0.9176 ± 0.002
Refractive index [η] ^{30°C}	1.487 ± 0.00	1.4877 ± 0.00
Optical rotation [α] _D ^{26°C}	-39.41° ± 0.01	-38.24° ± 0.04
Acid value (mg KOH/g)	7.71±0.31	8.53±0.14
Ester value (%)	34.37±0.19	37.26±0.81

Each value is the mean ± SD of three determinations. P<0.05 of independent sample T-test.

Chemical composition of the essential oil

The chemical compositions of China and Bangladeshi fresh ginger essential oils were identified and quantified by GC-MS and results are presented in **Table-4**. Twelve and ten compounds accounting to 99.99% and 99.91% were present in China and Bangladeshi fresh ginger variety respectively. China ginger essential oil characterized with high amount of monoterpene (33.02%) comprising 20.27% monoterpene hydrocarbon, 12.74% oxygenated monoterpene hydrocarbon and rest of 66.96% sesquiterpene hydrocarbon. The components zingiberene (38.102%), β-phellandrene (12.00%), β-sesquiphellandrene (9.546%), α-curcumene (9.224%), α-citral (7.571%), camphene (5.940%), farnesene (4.573%) and β-bisabolene (4.391%) were found the main components of the China ginger variety. On the other hand Bangladeshi fresh ginger essential oil characterized with high amount of monoterpene (55.19%) comprising with monoterpene hydrocarbon (37.156%), oxygenated monoterpene hydrocarbon (18.034%) and the rest of sesquiterpene hydrocarbon (44.72%). The major components were: zingiberene (26.587%), camphene (20.605%), β-phellandrene (9.921%), α-curcumene (7.90%), α-citral (9.758%), α-pinene (6.630%), borneol (5.395%), β-sesquiphellandrene (5.242%), and β-bisabolene (4.998%) while farnesene and germacrene-D were absent.

The current results of essential oil compositions of the two varieties are in good agreement with the findings of previous literature, where zingiberene reported the prominent constituents in the fresh ginger oil (Onyenekwe et al., 1999; Miyazawa et al., 1988; Malek et al., 2005; Singh et al., 2005; Sultan et al., 2005; Moshafi et al., 2009; Antonious et al., 2003). The chemical compositions of the ginger oil were reported on fresh and dried basis of the different varieties. Australian ginger oil was reported for their high citral contents (Purseglove et al., 1981) and the same was reported by Menut et al., (1994). El-Baroty et al., (2010) reported β-sesquiphellandrene, Singh et al., (2008) identified geranial, Agrawal et al., (2001) found curcumene and EL-Ghorab et al., (2010) reported camphene (15.9%-14.1%) as the prominent constituents in the fresh and dried ginger oil but the variation they found other constituents due to significant effect of drying. The variation of the essential oil composition could be the production and extraction condition, cultivars, variety, stage of mature, adaptive metabolism of plants, agro-climatic condition (climatic, seasonal, geographic) of the seasons and some other factors (Ghorab et al., 2010; El-Baroty et al., 2010; Singh et al., 2008). In the current experiment, the variation in the chemical compositions of the two varieties essential oil may be responsible for the said factors.

Table 4 Essential oil composition (% w/w) of Ginger varieties by GC-MS

Compounds	Composition (%)		
	R.I	China Ginger	Bangladeshi Ginger
Camphene ^a	943	5.940	20.605
α - Pinene ^a	948	2.337	6.630
β - Phellandrene ^a	964	12.00	9.921
Cineole ^b	1059	1.265	2.881
Borneol ^b	1138	3.91	5.395
α -Citral ^b	1174	7.571	9.758
β -Sesquiphellandrene ^c	1446	9.546	5.242
Farnesene ^c	1458	4.573	ND
Zingiberene ^c	1451	38.102	26.587
β -bisabolene ^c	1500	4.391	4.998
Germacrene D ^c	1515	1.137	ND
α -Curcumene ^c	1524	9.224	7.90
Total		99.99	99.91

RI: Retention Index, where minimum detection limit is 0.001%, ND stands for not detected. Superscript 'a' for monoterpene hydrocarbon, 'b' for oxygenated monoterpene hydrocarbon and 'c' for sesquiterpene hydrocarbon.

Antioxidant Activity (DPPH radicals)

The antioxidant activity of the different concentration of the fresh ginger essential oil was treated with DPPH radical and comparing with ascorbic acid and BHT. The results of these experiments are shown in Table-5. Ginger oil exhibited marked DPPH free radical scavenging activity in a concentration of 1.953-1000 μ g/mL. The IC₅₀ values of the two varieties were 61.18 \pm 0.75 μ g/mL and 56.71 \pm 0.45 μ g/mL with the inhibition of 78.49 \pm 0.16% and 80.77 \pm 0.25% of China and Bangladeshi fresh ginger variety respectively, Hence, the standard BHT and ascorbic acid (as positive control) showed the IC₅₀ value of 5.85 \pm 0.08 μ g/mL and 28.36 \pm 0.32 μ g/mL respectively. The highest activity was observed in Bangladeshi variety (80.77 \pm 0.25) at the 1000 μ g/mL dose level. Current results are almost comparable with previous reported data. El-Baroty et al., (2010) reported the IC₅₀=65.5 μ g/mL and Wei and Shibamoto (2007) reported the inhibition above 50% at a concentration of 200 μ g/mL. Antioxidants react with DPPH to convert it into 1,1-diphenyl-

2-picrylhydrazine. DPPH free radical scavenging activity is a concentration dependent manner. The degree of discoloration indicates the radical scavenging potential of the antioxidants (Singh et al., 2008). Lu and Foo (1995, 2001) reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidatives properties that create an effective defense system against free radicals. The antioxidant activities observed in ginger oil could be the synergistic effects of two or more compounds present in essential oil (Singh et al., 2008). Essential oil of ginger consists of a very complex mixture of various classes of organic compounds, which may produce either synergistic or antagonistic effects on the process of lipid oxidation. Moreover, the antioxidant activity might be responsible of some compounds such as camphene, cineole, borneol and zingiberene (Wei and Shibamoto 2007). Therefore, the antioxidative activity might be the such compounds which are present in appreciable amounts of the two varieties.

Table 5 DPPH scavenging activity of Ginger essential oil

Dose Concentration (μ g/mL)	Log Concentration (μ g/mL)	% of Inhibition (Mean \pm SD)			
		China Ginger	Bangladeshi Ginger	Ascorbic Acid(ASA)	BHT
1000	3.0	78.49 \pm 0.16	80.77 \pm 0.25	--	--
500	2.698	69.77 \pm 0.25	69.99 \pm 0.33	96.49 \pm 0.16	93.38 \pm 0.34
250	2.397	65.38 \pm 0.53	65.33 \pm 0.28	94.05 \pm 0.25	88.72 \pm 0.25
125	2.096	58.33 \pm 0.29	59.61 \pm 0.53	93.77 \pm 0.34	71.94 \pm 0.34
62.5	1.8	50.38 \pm 0.25	51.83 \pm 0.16	93.05 \pm 0.25	59.88 \pm 0.25
31.25	1.49	32.66 \pm 0.88	34.77 \pm 0.58	77.83 \pm 0.33	50.83 \pm 0.16
15.625	1.19	24.38 \pm 0.53	23.83 \pm 0.83	70.33 \pm 0.50	45.88 \pm 0.58
7.8125	0.89	15.88 \pm 0.78	21.77 \pm 0.25	56.05 \pm 0.41	37.33 \pm 0.44
3.9062	0.591	11.99 \pm 0.16	13.22 \pm 0.19	42.44 \pm 0.53	30.66 \pm 0.16
1.9531	0.290	11.83 \pm 0.28	12.27 \pm 0.75	39.66 \pm 0.57	26.33 \pm 0.60
IC₅₀ Value (μg/mL)		61.18\pm0.75	56.71\pm0.45	5.85\pm0.08	28.36\pm0.32

ASA = Ascorbic acid, BHT = tert-butyl-1-hydroxytoluene, Mean \pm SD, P<0.05 of independent sample T-test.

Antimicrobial Activity

According to the results of disk diffusion assay ginger essential oil are showed (Table-6) marked inhibitory effect against all tested strains with the zone of inhibition (15-20 mm) and comparable to ciprofloxacin (CF) as standard antibiotic. In this study *E.coli*, *S.paratyphi* and *S.dysenteriae* showed the highest activity (20 mm) in Bangladeshi variety than the China variety. On the other hand, *B.magesterium*, *B.subtilis*, *B.sereus* and *S.boydii* showed the minimum activity (15-16mm), Hence, *S.aureus*, *S.lutea*, *P.aureus* and fungi showed the same zone of inhibition (18mm) in the tested samples. Moreover, Bangladeshi ginger oil showed the overall highest inhibitory activity in the tested organism. The results indicate that different antimicrobial species showed different levels of sensitivities on the ginger essential oil and standard. There are several reports on the inhibitory effect of ginger oil on the growth of microorganism (Janes et al., 1999; Sing et al., 2008). Farag (1989) and Daw (1994) reported that chemical structure of essential oil or their main compounds such as the presence of functional polar group and aromaticity could play an important role for the antimicrobial activity. Therefore, the antimicrobial activity depends on major compound, chemical structure of the compounds and also synergistic action of the all constituents. It was reported that ginger oil rich in sesquiterpenoid compounds and therefore the compounds possessed a wide spectrum antimicrobial activity (Baratta et al., 1998; Anwar et al., 2009; El-bariti et al., 2010). In this study, the antimicrobial activity on ginger essential oil could be

presence of a major compound zingiberene and also predominant amount of sesquiterpenoid constituents.

Cytotoxicity Activity

Essential oil of *Z. officinale* of the two varieties are shown the most significant cytotoxic activity on brine shrimp larvae (Table-7). China and Bangladeshi ginger essential oil showed the LC₅₀ value 0.7951 and 0.8094 μ g/mL respectively in comparison to vincristine sulphate with the LC₅₀ value 0.4842 μ g/mL. No mortality was observed in 50 μ L/mL DMSO solution. Shariffar et al., (2009) and Moshafi et al., (2009) reported the significant activity found with the LC₅₀ value 10 μ g/mL and 0.0381 μ g/mL respectively in the concentration of 10, 100,1000 μ g/mL of the ginger essential oil. Moshafi et al., (2009) reported the cytotoxicity of the ginger essential oil is responsible for the major compounds zingiberene and β -sesquiphellandrene. The anti-tumor activity of compounds zingiberene and gingerol have been also reported (Chrubasik et al., 2005).

It would be inference that, the cytotoxicity of the essential oil of *Z. officinale* might be attributed to these compounds. As a part of our continuous research work, we have evaluated cytotoxicity of essential oil in the safety of herbal spices which have been used widely as condiment. At the high concentration level every flavoring agent or spice must be toxic. The results indicated that, the oil should be used as spice, flavouring in food or condiment in the certain concentration level.

Table 6 Antimicrobial activity of the ginger essential oil (400 µg/disc) and ciprofloxacin (CF) (5 µg/disc)

Test microorganisms		China (mm)	Bangladesh (mm)	CF (mm)
Gram positive bacteria	<i>Bacillus megaterium</i>	15	16	45
	<i>Bacillus subtilis</i>	15	16	46
	<i>Staphylococcus aureus</i>	18	18	46
	<i>Sarcina lutea</i>	18	18	45
	<i>Bacillus sereus</i>	16	15	45
Gram negative bacteria	<i>Escherichia coli</i>	18	20	46
	<i>Pseudomonas aureus</i>	18	18	46
	<i>Salmonella paratyphi</i>	18	20	46
	<i>Salmonella typhi</i>	17	18	45
	<i>Shigella dysenteriae</i>	18	20	46
	<i>Shigella boydii</i>	15	16	45
	<i>Vibrio mimicus</i>	16	18	46
	<i>Vibrio parahemolyticus</i>	16	18	46
Fungi	<i>Candida albicans</i>	18	18	45
	<i>Aspergillus niger</i>	18	18	46
	<i>Sacharomyces cereveaceae</i>	18	18	45

Table 7 Effect of vincristine sulphate and essential oil on shrimp naupli of *Ginger*

Log Dose (µg/mL)	% of mortality			Probit			LC ₅₀ (µg/mL)		
	VcS	China	Bangladesh	VcS	China	Bangladesh	VcS	China	Bangladesh
-1.1079	10	10	10	3.72	3.758	3.743	0.4842	0.7951	0.8094
-0.8069	20	20	20	4.16	4.165	4.161			
-0.5051	50	40	40	5.00	4.750	4.752			
-0.2041	60	60	50	5.25	5.252	5.000			
0.0969	70	60	60	5.52	5.253	5.253			
0.3979	80	70	70	5.82	5.524	5.524			
0.699	90	70	80	6.25	5.498	5.841			
1	100	80	80	7.33	5.809	5.790			
1.301	-	90	90	-	6.274	6.258			
1.6021	-	100	100	-	7.158	7.229			

Elemental composition

The elemental compositions of *Z. officinales* of the two varieties are organized in essential and non essential elements and shown in **Table 8(a)** and **Table 8(b)** respectively. The concentrations of the elements are calculated on fresh (FB) and dry weight basis (DB) within the significance level ($p \leq 0.05$).

Essential elements

The mineral contents of Na (100.04 mg/kg), K (6.32 g/kg), Mg (0.56 g/kg) and Ca (0.472 g/kg) were found the highest in the Bangladeshi ginger variety than the China variety, while Ca (0.509 g/kg) was the highest in China variety on fresh weight basis. In comparison to published elemental data, Na (66.0 mg/kg DB) (Ismail et al., 2011), K (21723±19 mg/kg DB, 2248 mg/kg DB and 4150 mg/kg FB) (Olabanji et al., 2007; Sheng-bang et al., 2005; USDA 2011), Mg (600 mg/kg DB, 22.4 mg/kg FB and 1613 mg/kg DB) (Famurewa et al., 2011; Ismail et al., 2011; Sheng-bang et al., 2005), Ca (1100 mg/kg DB and 160 mg/kg DB) (Famurewa 2011; USDA 2011) and Fe (32.0 mg/kg DB, 6 mg/kg FB, 34.0±1.1 mg/kg DB and 135.2 mg/kg DB) (Ismail et al., 2011; USDA 2011; Ozkutlu et al., 2006; Sheng-bang et al., 2005) were lower than the current findings. On the other hand, the reported values of Na (130 mg/kg FB) (USDA 2011), Mg (3217±48 mg/kg FB), Ca (3337±42 mg/kg FB) and Fe (663±4 mg/kg DB) (Olabanji et al., 2007) were higher than the current results. These mineral elements play a significant role in human metabolism and life process.

The elements of Be (1.27), Ga (12.04), Al (79.23), Se (408.79), Ag (3.22) and Cs (3.94), were observed the highest amounts in the Bangladeshi ginger variety, whereas Li (68.74), Co (7.79) and Bi (3.93) were found the highest in the China variety in µg/kg on fresh weight basis. Hence, Ni was not found in the China variety. A comparisons with reported data, Co (1.073, 0.480 mg/kg DB) (Ismail et al., 2011; Lamari et al., 2011), Ni (0.06, 6.7±1.07, 2.37±0.61 mg/kg DB) (Ismail et al., 2011; Olabanji et al., 2007; Ziarati et al., 2012) and Be (32.7 ±11.7 mg/kg DB) (Olabanji et al., 2007) contents were higher than the current experimental results. In the case of Se contents (188.97 and 408.79 µg/kg FB) which are interestingly higher than the USDA (2011) specification (7.0 µg/kg FB). Se has got anti-oxidising function and it is essential for providing the organism with triiodothyroxine produced from thyroxine (Forrer et al., 1998). It contributes to the maintenance of cellular antioxidative balance when taken up at the recommended dietary allowance of 50-100 µg/day and tolerable upper intake limit is 400 µg/day (FNB 1989). The high dosage of selenate has been

shown to normalize hyperglycemia (Chowdhury et al., 2007). The current values of Al were found higher than the reported (0.22 mg/kg DB) (Alam et al., 2008) value. The importance of Co is the component vitamin B12. The Daily Dietary Intake (DDI) of Co is 7-50 µg/day and the maximum level is 0.3 mg (Lesnicewez et al., 2006). Ni plays an important role in the production of insulin and mostly present in the pancreas. The recommended daily intake should be less than 1 mg/day beyond which it is toxic (Ziarati et al., 2012). The elements of Li, Be, Ga, Ag, Cs and Bi were found the first time in Bangladeshi sample by ICP-MS. These elements play important role in the physiological functioning in the body. These elements are essential constituents of enzymes and play a vital role in human metabolism. The human body cannot synthesize them and they must be supplied by food. Essential elements in spices may be correlated with their taste and adding spice in food not only increase flavor but also enhance its nutritive value by providing several essential nutrients in bioavailable form (Ila and Jagun 1980; Sing et al. 2006). However excessive intake limit is the cause of the toxic effect in the body.

Non-essential elements

In recent years, much emphasis is being laid on Non-essential or toxic elemental contents, in several countries in respect of spice and medicinal plants. **Table-8(b)** showed the toxic elemental contents of the Bangladeshi and China variety. As (45.61) and Pb (101.99) contents were present higher amount in the Bangladeshi variety than the China variety (µg/kg FB). On the other hand Cd (7.75) and Hg (113.86) contents were found higher in China variety than Bangladeshi variety in µg/kg on fresh basis. Cd 0.072±4.1, 0.12±0.01, 1.64±0.11 mg/kg DB (Ozkutlu et al., 2006; Alam et al., 2008; Ziarati et al., 2012), Pb 7.06±0.21 and 6.0 mg/kg DB (Ziarati et al., 2012; Sheng-bang et al., 2005) and Hg 0.58±0.06 mg/kg DB (Ziarati et al., 2012) were also found in the earlier reported data. Pb is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration (ATSDR 2007). Cd intoxication can lead to kidney, bone and pulmonary damages (Godt et al., 2006). Hg poisoning can result in damage to the brain, kidney, and lungs, including acrodynia (pink disease), Hunter-Russell syndrome and Minamata disease (Jack et al., 2007). The acute sign of As poisoning includes fever, anorexia, hepatomegaly, cardiac arrhythmia, transient encephalopathy and irritation of the gastrointestinal tract (Anonymous 1998). The WHO maximum limit for Pb and Cd prescribed in herbal medicines and products is 10 mg/kg and 0.3 mg/kg while the dietary intake limits are 3 and

10.3 mg/kg respectively (WHO 1998). On the other hand the permissible limits of Hg and As are 1 and 10 mg/kg in foodstuff respectively (FDA 1999). The results of toxic elemental contents were found below of the permissible limit throughout the study.

Element uptaken by a plant is its characteristic property and may depend on the use of fertilizers, irrigation water and different climatologically or geo-environmental factors. (Chowdhury et al., 2007). In the current study, the elemental composition of ginger vary from one variety to other due to the said factors.

Table 8(a) Essential elemental composition of Ginger variety

Elements ($\mu\text{g}/\text{kg}$)	China		RSD %	Bangladesh		RSD %
	DWB	FWB		DWB	FWB	
Li	379.78	68.74	2.36	207.35	47.05	2.01
Be	3.67	0.66	18.82	5.60	1.27	17.31
Co	43.04	7.79	1.28	23.69	5.38	0.35
Ni	ND	0	ND	558.58	126.74	0.28
Ga	51.13	9.25	0.48	53.09	12.04	1.69
Ag	6.47	1.17	0.79	14.16	3.22	0.73
Cs	12.48	2.26	5.71	17.35	3.94	1.57
Bi	21.69	3.93	95.27	9.91	2.25	2.36
Se	1044.06	188.97	11.54	1801.62	408.79	6.12
Na (mg/kg)	474.13 \pm 0.6	85.82 \pm 0.10	-	440.19 \pm 0.8	100.04 \pm 0.18	-
Fe (mg/kg)	215.04	38.92	0.1	195.55	44.37	0.1
Al (mg/kg)	269.18	48.72	0.1	349.20	79.23	0.1
K (g/kg)	16.97 \pm 8.6	3.07 \pm 1.6	-	27.86 \pm 12.6	6.32 \pm 2.85	-
Ca (g/kg)	2.81	0.509	0.1	2.08	0.472	0.1
Mg (g/kg)	2.762	0.49	0.1	2.476	0.56	0.1

ND: Not Detected, RSD: Relative Standard Deviation, $p \leq 0.05$.

Table 8(b) Non-essential elemental composition $\mu\text{g}/\text{kg}$ of Ginger variety

Elements	China		RSD %	Bangladesh		RSD %
	DWB	FWB		DWB	FWB	
As	55.66	10.07	89.47	201.03	45.61	1.09
Hg	629.07	113.86	0.1	355.52	80.67	0.1
Pb	331.61	60.02	32.77	449.49	101.99	1.69
Cd	42.80	7.75	2.77	14.67	3.33	1.36

RSD: Relative Standard Deviation, $p \leq 0.05$.

CONCLUSION

Ginger is usually mixed with tea, beverage and also used as spice for flavoring purposes all over the world. The essential oil composition of ginger variety showed rich in sesquiterpene and monoterpene hydrocarbon compounds. The biological studies of the oil possessed the high antioxidative activity as well

as antimicrobial effect. This may be used in food industries as preserver of food product against spoilage by bacteria and fungi. Nineteen elements were detected at various concentration levels. These elements are the meaningful sources of major and trace elements in the human nutrition. The proximate and mineral compositions of Bangladeshi and China ginger in respect of their nutritional values are in good agreement with USDA quality standard. On the other hand, toxic elemental compositions were found below the recommended toxic level, whose consumption is not harmful as per recommended doses. The results of these studies imply that Bangladeshi ginger variety complies with the

international quality standard and it may be used as spice and in food industries as well as in medicinal preparation.

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